

Organic Chemicals from Biomass

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CRC Press, Inc.
Boca Raton, Florida

1981

Chapter 3

BIOCONVERSION OF AGRICULTURAL BIOMASS TO ORGANIC
CHEMICALS

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I. INTRODUCTION

This article will deal primarily with the current methods available to generate organic chemicals via fermentation from crop biomass, starch materials, agri-residues, and agro-industrial wastes. A comprehensive analysis of the characteristics and availability of agri-residues and industrial wastes is available and will be identified by other authors contributing to this subject. Relative composition of biomass, residues, and waste materials will be identified only when necessary to define substrates for production of specific chemicals through fermentation. Extensive studies on the utilization of animal products and animal waste management by Loehr¹ cover research conducted in the past 15 years. Overviews by Sloneker et al.^{2,3} on crop residues and animal wastes defines the availability of these resources in the U.S. A more recent review by Detroy and Hesseltine⁴ deals mainly with both chemical and microbiological conversion of crops and agri-residues to useful by-products, i.e., animal feed supplements, biopolymers, single-cell protein, methane, and chemical feedstocks.

II. IDENTIFICATION AND POTENTIAL OF BIOMASS AND AGRICULTURAL RESIDUES

Increasing attention has been noted to the possibilities of utilizing photosynthetically active plants as natural solar energy-capturing devices, with the subsequent conversion of available plant energy into useful fuels or chemical feedstocks, such as alcohol and biogas, via fermentation. Acquisition of biological raw materials for energy capture follows three main approaches: (1) purposeful cultivation of so-called energy crops, (2) harvesting of natural vegetation, and (3) collection of agricultural wastes. Lewis⁵ has recently described the energy relationships of fuel from biomass in terms of net energy production processes (Table I). Table I presents data in terms of energy requirements, net energy gains and losses, and land area equivalents for a number of relevant conversion systems. Starch crops like cassava and other saccharide plants, notably sugar cane, appear to be the most favorable in terms of energy balance. More technological innovations would be required to derive a favorable energy balance for the conversion of the lignocellulosic raw materials owing to the energy intensive pretreatment requirements to render the substrate fermentable.

Biomass, or chemical energy, can serve as an energy mechanism to be harvested when needed and transported to points of usage. Land availability must be carefully evaluated in view of the potential of this energy alternative.

Since energy deficits are enormous, significant sources of biomass must be acquired. Some 95% of the field crops are planted for food grains. Since the majority of the plant residues (stalks and straw) are unused after harvest, these residues are potentially available for collection and conversion to useful energy.

The potential annual supply of U.S. cellulosic residues from domestic crops is certainly in excess of 500 million tons (dry weight). In general, cereals produce some 2 lb of straw per pound of grain harvested. Significant accumulations of major crop residues are, of course, confined to those areas of intensive cropping. The general distribution of potentially collectible cereal straws in the U.S. is depicted in Figure 1. All crops produce collectible residues; however, the distribution of straw residues increases the costs of utilization. These collectible residues from major and minor crops are depicted in Tables 2 and 3. The residues produced by the majority of these crops are left in the fields after harvest. Only with sugar cane, vegetables, fruit, and peanuts are there significant accumulations at specific processing sites.

Since the quantity of straw produced is equal to or greater than the quantity of

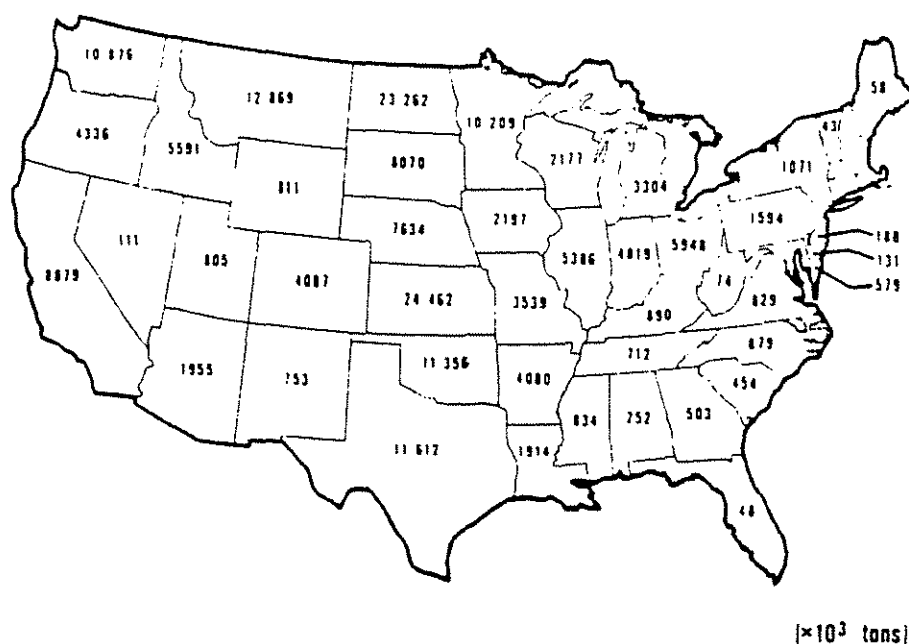


FIGURE 1. Geographical distribution of cereal straws (flux, wheat, rye, rice, oats, and barley).

Table 1
ENERGY REQUIREMENTS, NET ENERGY GAINS AND
LOSSES, AND LAND AREA EQUIVALENTS FOR A
NUMBER OF CONVERSION AND PRODUCTION SYSTEMS

Principal substrate	Product	GER product (GJ/t)	Net energy	
			(GJ/t)	(GJ/ha/yr)
CO ₂	Energy crops	1.26	+ 16	+ 1090
Raw sewage	Algae*	57	-34	-850
Raw sewage	Algae*	18	+ 5	+ 125
Algae	Methane*	168	-112	-627
Livestock waste (UK)	Methane	144	-88	-0.88
Sugar cane	Ethanol	24	+ 3	+ 51
Cassava	Ethanol	61	34	-71
Timber	Ethanol	239	-212	-74*
Timber	Ethanol*	98	-71	-16*
Straw	Ethanol	222	-195	-138

- * The figures relate to current methods adopted.
- * The figures are estimates of what should be possible at present.
- * Cellulose hydrolyzed to fermentable sugars by fungal enzymes.
- * Figures expressed on basis of land area requirement to annually replenish the quantity of wood substrate used.
- * Cellulose hydrolyzed to fermentable sugars by acids. Also requires 470% man-power increase over enzyme route.

edible grain from cereal crops, its utilization is of paramount importance. Present constraints on the utilization of cereal by-products include: new technology development, residue collection, marketability, practical utility of residues, and research on

Table 2
MAJOR CROPS—CURRENT ESTIMATES

Commodity	Acres harvested ($\times 10^6$)	Tons/acre	Residue (dry wt)	
			Total $\times 10^6$	
			Minimum	Maximum
Corn	65	2—3	130	195
Hay	64	3—7	192	448
Soybeans	60	1—2	60	120
Wheat	60	1—2	60	120
Sorghum	16	2—3	32	48
Oats	14	1—2	14	28
Cotton	12	1—2	12	24
Barley	11	1—2	11	22
Total	302	—	319*	557*

* Total yields do not include hay crop.

Table 3
MINOR CROPS—CURRENT ESTIMATES

Commodity	Acres harvested $\times 10^6$	Tons/acre	Residue (dry wt)	
			Total $\times 10^6$	
			Minimum	Maximum
Vegetables	3.5	1—2	3.5	7.0
Fruit	3.3	1	3.3	3.3
Rice	2.2	1—2	2.2	4.4
Flax	1.8	1	1.8	1.8
Peanuts	1.5	1—2	1.5	3.0
Sugar beets	2.0	1—2	2.0	4.0
Sugar cane	1.5	6—10	9.0	15.0
Rye	1.0	1—2	1.0	2.0
Total	16.8	—	24.3	40.5

model bioconversions. Collection costs of important residue resources govern the economic feasibility of bioconversion processes for fermentation chemicals.

Mechanical equipment exists for harvesting corn refuse, silage, or hay, and can be readily be used for the collection and hauling of plant residues to central locations for processing. Sloneker² discusses types of harvesting operations that can be employed to stack, bail, windrow, chop, and transport various crop residues. Time and expensive equipment are serious deterrents to collection of crop refuse in on-the-farm operations. Any major increase in the use of cereal straws and other residues will require major efforts to collect, handle, transport, and deliver at a central location or plant so that they will be competitive with other raw materials for chemical production. Benefits from mass collection of straw residue must be balanced against the consequences of its removal from fertile crop land. Residues plowed under or left on the surface (conservation tillage) increase the tilth of the soil, aid in H_2O sorption, and reduce soil erosion; therefore, the impact that continuous residue removal will have on soil fertility must be thoroughly examined. Refractory material that remains after bioconversion of agro-residues may, if returned to the land, provide sufficient organic matter in the soil for tilth.

Table 4
GRAIN PROCESSING WASTE CHARACTERISTICS*

Parameter	Corn wet milling (average)	Corn dry milling (average)
Flow ^a	18.3	—
Biological Oxygen Demand (BOD)	7.4	1.14
Chemical Oxygen Demand (COD)	14.8	2.69
Suspended solids	3.8	1.62

* Corn wet milling, to produce corn syrup or starch. Corn dry milling — to produce meal and flour, water usage limited to washing, tempering, and cooling.

^a Flow = 1/4 kg grain processed. BOD and suspended solids = kg/kg grain processed.

From Development Document for Effluent Limitations Guidelines and New Source Performance Standards for the Grain Processing Segment of the Grain Mills Point Source Category, EPA 440/1-74-028a, Environmental Protection Agency, Washington, D.C., 1974.

The wet-milling process of cereal grains produces considerable quantities of grain carbohydrate waste. The waste-liquid streams that arise as a result of steeping, corn washing, grinding, and fractionation of corn yield cornstarch, corn syrup, gluten, and corn steep liquor. Increased studies are necessary on the bioconversion of these negative value carbohydrate wastes into alcohol, C_2 and C_4 chemicals, and methane, as well as on economical pretreatment of the industrial waste being produced. A summary of waste characteristics from grain processing is depicted in Table 4. No process wastewaters are produced by the milling of wheat and rice grains. However, the bran from these two cereals contains 5 to 10% oil and is rich in certain B vitamins and amino acids.

A major potential resource of the immense animal industry in the U.S. is the annual generation of over 2 billion tons of waste. Recent changes in the fertilizer and animal-feeding industries have resulted in the accumulation of animal wastes into localized areas. This localization has produced air and water pollution problems. Technological changes in large-volume cattle feeding have created a serious need for new waste technology, either through cost reductions in handling to eliminate pollution hazards or some type of bioconversion process to useful fuels or chemical feedstocks.

The utilization of animal wastes, other than land usage, as a waste management alternative has proceeded in two main areas: biological and thermochemical. Major experimentation has involved methane formation, single-cell protein production, and microbial fermentation and refeeding. Animal wastes are excellent nutrient sources for microbial development. Major constituents are organic nitrogen (14 to 30% protein), carbohydrate (30 to 50%, essentially all cellulose and hemicellulose), lignin (5 to 12%), and inorganic salts (10 to 25%).

In most biological processes, microorganisms consume nutrients present in the wastes to increase their own biomass and, through substrate utilization, release various gases and other simple carbohydrate materials. There are mainly two classes of biological processes: biogas (or an anaerobic fermentation) and biochemical hydrolysis. The biochemical processes produce primarily protein, sugar, and alcohol, whereas the anaerobic fermentation takes place under an oxygen-deficient environment to produce methane.

All of these processes have been successfully demonstrated for livestock manure.⁶

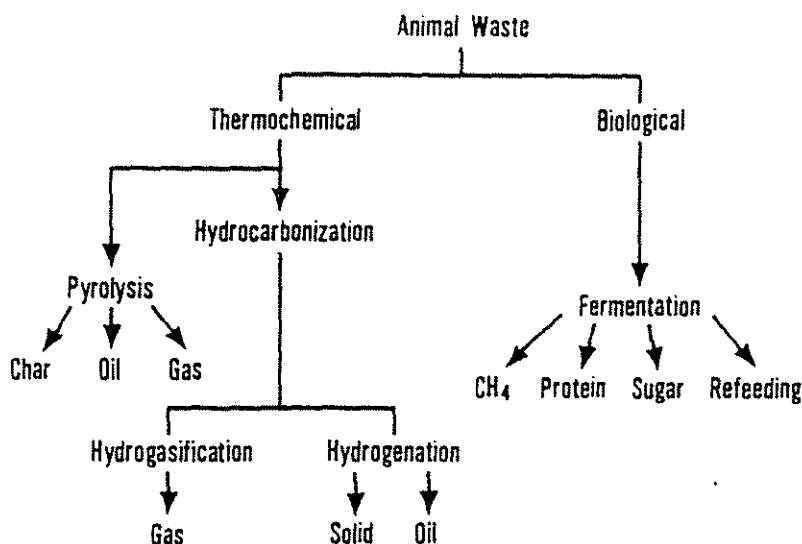


FIGURE 2. Process alternatives for the generation of fuels from animal waste.

Table 5
MANURE PRODUCTION IN
THE UNITED STATES*

Animal*	Dry measure × 10 ⁶ t	Percent of total
Cattle	210	81.1
Swine	25	9.7
Horses	14	5.4
Poultry	6.2	2.4
Sheep	3.1	1.2
All	258.3*	100

* Wet weight = 1.5×10^6 t at 16.1% dry matter.

The various biological and chemical processes alternatives for the generation of renewal fuels and chemicals from animal manure is depicted in Figure 2. Total production of manure in the U.S. according to classes of animals and relative concentrations to the total, is shown in Table 5.⁷⁻⁹

The utilization of sugar cane bagasse must be considered on a country-by-country basis. Bagasse is the fibrous residue obtained after the extraction by crushing of sugar cane stalks. This roller-mill process removes 95% of the sucrose, producing a residue that contains some 50% moisture and consists of 15% lignin and 75% cellulose. Annual world production of bagasse is greater than 100 million tons. Bagasse has been used mainly as a fuel in sugar cane factories, for production of pulp and paper, and for structural materials. Extensive research has been conducted in the past few years on bagasse as a cellulosic raw material for single-cell protein production.^{10,11} Cellulosic wastes, such as bagasse, have also received considerable attention as resource material for chemical processes and energy conversions (anaerobic fermentation to methane or ethanol).

The largest wastes from dairy food plants are whey from cheese production and

Table 6
RAW WASTE LOADS* FOR THE FRUIT AND
VEGETABLE PROCESSING INDUSTRY

Category	Flow (gal/ton)	BOD (lb/ton)	Total suspended (lb/ton solids)
Fruit			
Apple processing	690	4.1	0.6
Apple products, except juice	1,290	12.8	1.6
Citrus, all products	2,420	6.4	2.6
Olives	9,160	87	15
Pickles, fresh packed	2,050	19	4
Tomatoes	2,150	8	12
Peeled products	1,130	3	5
Vegetables			
Asparagus	16,530	4.2	6.9
Beets	1,210	39.4	7.9
Carrots	2,910	19.0	24
Corn			
Canned	1,070	28.8	13.4
Frozen	3,190	40.4	11.2
Lima beans	6,510	27.8	20.7
Peas			
Canned	4,720	44.2	10.8
Frozen	3,480	36.6	9.8
White potatoes	1,990	54.6	74.8

- * The raw waste load is in terms of the quantity of wastewater parameter per ton of raw material processed for fruits and vegetables. Raw waste loads are those generated from canning processing.

pasteurization water. A pound of cheese produces 5 to 10 lb of fluid whey with a biological oxygen demand (BOD) of 32 to 60 g/l, depending upon the process. Whey is an excellent nutrient source for microbe development, containing 5% lactose, 1% protein, 0.3% fat, and 0.6% ash.

Processing plant wastes for different fruits and vegetables vary in character and quantity. The effluents consist primarily of carbohydrates, starches and sugars, pectins, vitamins, and plant cell-wall residues. One must consider how the various processing operations affect availability and type of residues. Table 6 depicts some typical fruit and vegetable residues and characteristics based upon the quantity of material processed or quantity of material produced. Supply problems, due to various geographical locations and seasons, hinder large-scale utilization of these residues for fermentation purposes. Waste-waters and peels from potato processing also serve as an excellent starch source, but seasonal production hinders utilization of residues. The most promising end uses for potatoes involve recovery of starch for cattle feeding and for production of sugar, single-cell protein, and biogas.

The enormous amounts of spoiled, damaged, and culled fruits and vegetables are excellent sources of carbohydrate material. These materials typically are good substrates for the growth of many fungi, especially on acid fruits. However, a real problem exists in that these materials are seasonal, so that a microbial process cannot be run the year around because large amounts are available only at certain times.

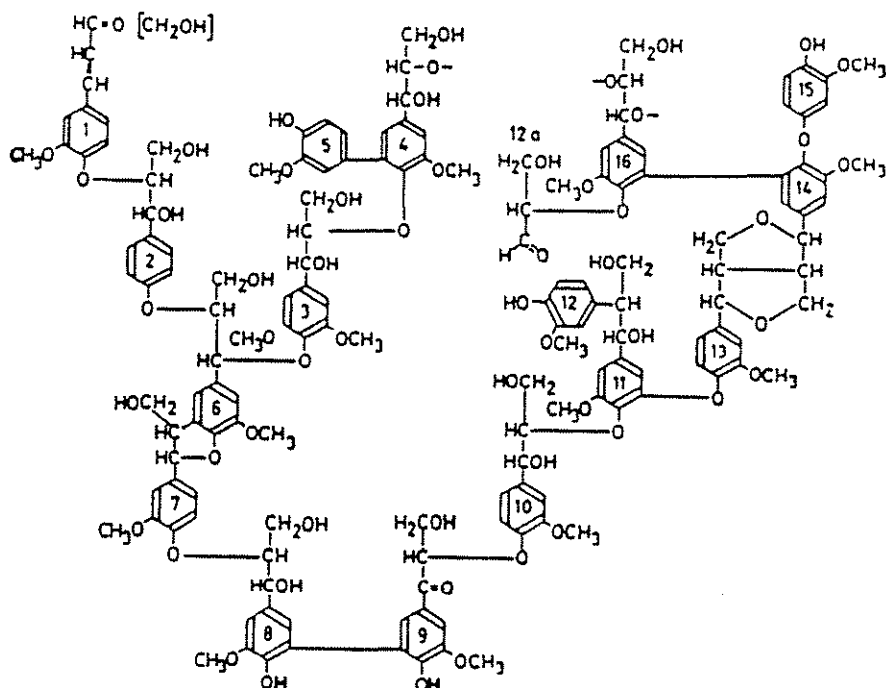


FIGURE 3. The structure of lignin.

III. COMPOSITION OF AGRI-COMMODITIES

The major components in agricultural residues are the structural cell-wall polysaccharides, primarily cellulose and hemicellulose. The latter two are the most plentiful renewable resource produced by most green plants. These carbohydrates constitute 45 to 70% of the weight of a dried plant, varying according to age and maturity of plant at harvest. Pure cellulose, such as cotton fiber, is rarely found in nature, but rather in combination with other polymers such as lignin, pectin, and hemicellulose. Lignin comprises from 3 to 15% of the dried plant residue. This material is the structural glue that binds filaments of cellulose into fibers for cell integrity and rigidity. Lignin is found in all fibrous plants, and generally increases with age of the plant. Cellulose increases in aging fibrous plants with a decrease in soluble sugars and an increase in lignin. Lignin is a three-dimensional polymer formed by the condensation of cinnamyl alcohol monomers depicted in Figure 3. All possible combinations of the cinnamyl radicals can occur, resulting in various types of bonding. The exact linkage and structure of the lignin-cellulose complex is of considerable debate. There is considerable intermolecular bonding between the uronic acids of hemicellulose and lignin phenolic groups. Lignin apparently forms a three-dimensional net around the cellulose fibers. It is in this fashion that the complex cellulose is rendered unavailable to subsequent enzyme degradation. It is also in this complex area of lignin-cellulose interaction where the ultimate utility of agro-residues has its future. Chemical and/or biological modification of this lignocellulosic complex would result in increased digestibility of the agro-residue, increased hydrolysis rates, and saccharification. Continued research in the area of utilizing lignocellulosics is of paramount importance to the future of these negative value carbohydrate wastes. Table 7 depicts the relative composition of some important U.S. agro-residues.

Table 7
COMPOSITION OF AGRICULTURAL RESIDUES

Plant residue	Carbohydrate (%)							Lignin (%)	Protein (%)
	Arabinose	Xylose	Mannose	Galactose	Glucose	Total	Cellulose		
Corn stalks	1.9	15.5	0.6	1.1	37.7	56.8	29.3	3.1	5.5
Flax straw	2.1	10.6	1.3	2.2	34.7	50.9	34.5	—	7.2
Kenaf stalks	1.5	12.8	1.6	1.3	41.4	58.6	41.9	12.3	4.6
Soybean straw	0.7	13.3	1.7	1.2	43.7	60.6	41.4	—	5.5
Sunflower stalks	1.4	19	1.35	0.05	39.4	43.8	35.1	—	2.1
Sweet clover hay	3.2	7.2	1.2	1.7	31.1	44.4	29.8	—	24.7
Wheat straw	6.2	21.0	0.3	0.6	41.1	69.2	40.0	13.6	3.6
Cattle waste	0.38	0.77	0.73	0.97	24.4	27.2	16.4	6.5	10.1
Swine waste	0.43	0.83	0.98	1.27	25.5	29.8	16.6	1.6	15.1

IV. TECHNOLOGIES FOR UTILIZATION OF RESIDUES

Residue utilization must be considered with optimism due to the large quantities of wastes and by-products available, the need to better utilize existing resources, and the successful processes that have been attained. Successful residue utilization must include the following changes in approach:

1. Residues as resources, not wastes
2. Incentives to change philosophy
3. Evaluation of socioeconomic aspects
4. Use of appropriate technology
5. Beneficial use
6. Proper market
7. Better usage of raw materials

Promising technologies are needed for the utilization of agricultural and agro-industrial residues. Some of the most promising and successful technological processes for the utilization of agro-wastes are described in Table 8.

V. CHEMICALS FROM CARBOHYDRATE RAW MATERIALS

Recent progressive increases in the cost of crude oil have resulted in considerable attention being focused upon fermentation technology. The major production of industrial alcohol and of C_3 and C_4 chemicals is derived from fossil fuels. Alternative process routes for the production of organic chemicals involve fermentation primarily through bioconversion of carbohydrate raw materials to chemicals. Tong¹² has recently described fermentation routes for the production of C_3 and C_4 chemicals from specific available raw materials.

The major organic chemicals that are produced from carbohydrate raw materials by microbial fermentation are identified in Table 9. The main carbohydrate sources for fermentation are as follows:

1. Starch grains from corn, wheat, barley, and other cereals
2. Sucrose from beet, cane, and sorghum
3. By-product materials from processing, i.e., fruit and vegetable wastes, starch streams from milling grains, cattle feedlot waste, dairy whey, molasses, and distiller grain

The various chemicals produced via fermentation will be discussed individually in terms of yield, substrate resource, and future opportunities as alternative resource and feedstock chemicals.

VI. CONVERSION OF BIOMASS TO SUGAR

As mentioned previously, the bioconversion of plant biomass to fermentation chemicals depends upon the basic structural composition and integrity of lignocellulose. Most lignocellulosic plant materials require some preliminary biological and/or chemical pretreatment before a direct fermentation to ethanol or other chemicals can be investigated. In general, before a microbial fermentation can be contemplated, the plant polymers, whether lignocellulosics, hemicellulose, or starch, must be hydrolyzed to simple sugars for utilization.

Table 8
TECHNOLOGIES AVAILABLE FOR UTILIZATION OF AGRO-INDUSTRIAL AND
AGRICULTURAL WASTES

Residue substrate	Process	Product	Operational*	
			Advantages	Disadvantages
Animal waste	Microbial	CH ₄ , feed supplement	Cheap resource, produces energy, available, reduce pollution	High initial investment
Animal waste	Microbial	Cattle refeeding, single-cell protein		
Sugar cane bagasse	Microbial	Single-cell protein	Surplus availability, technology available	
Dairy whey	Microbial	Single-cell protein, alcohol	Reduce pollution, surplus availability	High salt content, transportation
Cereal process waste	Microbial	Single-cell protein	Reduce BOD and COD	
Cellulosic pulps	Enzymatic (saccharification)	Sugar		
Hemicelluloses (xylans)	Enzymatic	Xylose		Expensive
Starch waste	Microbial	Alcohol	Cheap resource	
Wood pulp sulfite liquor	Microbial	Single-cell protein	Surplus availability	

* Items listed on basis of economics, availability, pollutant, and source.

Table 9
CHEMICALS FROM FERMENTATION PROCESSES

Chemicals	Structure	Produced by
Ethanol	$\text{CH}_3\text{CH}_2\text{OH}$	Species of <i>Saccharomyces</i>
<i>n</i> -Butanol	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$	<i>Clostridium acetobutylicum</i>
2,3-Butylene glycol	$\begin{array}{c} \text{OH} \quad \text{OH} \\ \quad \\ \text{CH}_3\text{CH}-\text{CH}-\text{CH}_3 \end{array}$	Species of <i>Aerobacter</i> and bacilli
Glycerol	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{HC}-\text{OH} \\ \\ \text{CH}_2\text{OH} \end{array}$	Species of <i>Saccharomyces</i>
Acetic acid	CH_3COOH	<i>Clostridium thermoaceticum</i> <i>Acetobacter</i> species
Acetone	$\begin{array}{c} \text{O} \\ \\ \text{H}_3\text{C}-\text{C}-\text{CH}_3 \end{array}$	<i>Clostridium acetobutylicum</i>
Isopropanol	$\begin{array}{c} \text{OH} \\ \\ \text{CH}_3\text{CHCH}_3 \end{array}$	Species of <i>Clostridium</i> and bacilli
Fumaric acid	$\begin{array}{c} \text{COOH} \\ \\ \text{CH} \\ \\ \text{CH} \\ \\ \text{COOH} \end{array}$	Species of <i>Rhizopus</i> and <i>mucor</i>
Succinic acid	$\begin{array}{c} \text{CH}_2\text{COOH} \\ \\ \text{CH}_2\text{COOH} \end{array}$	Species of <i>mucor</i> , <i>Rhizopus</i> , <i>Fusarium</i>
Citric acid	$\begin{array}{c} \text{COOH} \\ \\ \text{CH}_2 \\ \\ \text{HO}-\text{C}-\text{COOH} \\ \\ \text{CH}_2 \\ \\ \text{COOH} \end{array}$	<i>Aspergillus niger</i> , <i>Candida lipolytica</i>
Lactic acid	$\begin{array}{c} \text{CH}_3 \\ \\ \text{HC}-\text{OH} \\ \\ \text{COOH} \end{array}$	Species of <i>Rhizopus</i> and <i>mucor</i> , lactobacilli
Propionic acid	$\text{CH}_3\text{CH}_2\text{COOH}$	Species of propionibacterium
Malic acid	$\begin{array}{c} \text{COOH} \\ \\ \text{HO}-\text{CH} \\ \\ \text{CH}_2 \\ \\ \text{COOH} \end{array}$	<i>A. niger</i> , <i>A. itaconicus</i> , <i>Proteus vulgaris</i>
Methanol	CH_3OH	Species of <i>Methylomonas</i> , <i>Pseudomonas</i> , <i>Methylococcus</i>

Lignin and cellulose crystallinity are the two major deterrents to the effective utilization of lignocellulosic residues for chemical, enzymatic, and microbiological conversion processes to available sugars. The lignin polymer severely restricts enzymatic and microbial access to cellulose. Millet and co-workers¹¹ have published most comprehensive reviews on specific physical-chemical pretreatments for enhancing cellulose saccharification. These pretreatment steps are applicable to a wide variety of lignocellulosic materials that can be delignified to varying extents depending upon the type of pretreatment methods. Ladisch and co-workers¹⁴ have recently described an organic solvent pretreatment process of cellulosic residues, followed by a cellulase hydrolysis step, to yield quantitative saccharification of the α -cellulose to simple sugars. The process yields 90 to 97% conversion of the cellulose of agri-residues to glucose. Successful application of this type of saccharification technology opens up new horizons to the utilization of biomass as a source of fuels, chemicals, and food.

The other type of pretreatment step of lignocellulosics centers around biological delignification. Kirk¹⁵ has published a most comprehensive review on microorganisms that effect biological lignin degradation. More recent work on the physiological role white-rot fungi play in degradation of lignins draws attention to the synthesis of ¹⁴C-labeled lignins and lignocelluloses as specific substrates for microorganisms. Crawford et al.^{16,17} and Kirk et al.¹⁸ discuss recent work on the degradation of labeled lignins and lignocelluloses by fungi and actinomyces to ¹⁴CO₂. These techniques will become invaluable to the study of biodelignification and the role microbes may play in modifying lignocellulosics for subsequent saccharification.

VII. FERMENTATION CHEMICALS: ANAEROBIC AND AEROBIC

The anaerobic products of microbial metabolism consist of various organic solvents, i.e., acetone, ethanol, *n*-butanol, and isopropanol. Fermentations do not require aeration, and product recovery is accomplished through conventional distillation recovery methods. Fermentation processes to produce these chemicals are not dependent upon pure carbohydrate resources, but can utilize any type of pentose and/or hexose stream generated from biomass feedstocks.

Fumaric acid, glycerol, and 2,3 butylene glycol represent the main chemicals of aerobic fermentation. Tong,¹² in a recent review, discusses the production of various C₁ and C₂ chemicals and the current energy costs of production via fermentation. The aerobic processes are energy-intensive and require cooling, aeration, and agitation since these processes are highly exothermic due to carbohydrate oxidation. A comparison of attained vs. theoretical weight yields on dextrose for the fermentation products mentioned is depicted in Table 10. The major process used exclusively before 1950 was the acetone, *n*-butanol, and ethanol fermentation. Some improvement has been shown in this process. However, fermentation-derived solvents are presently only a minor factor in North America, although significant quantities are being produced in countries such as South Africa, where inexpensive fermentation resources are available.

In 1976, the total U.S. production of nine C₁ and C₂ chemicals, including ethanol, was near 4 million tons. Only 2% of these chemicals is presently derived via fermentation. Only butanol, acetone, fumaric acid, and ethanol are currently produced from both petroleum and carbohydrate feedstocks. The estimated percentage of organic chemicals produced by fermentation is depicted in Table 11.

Tong¹² indicates that in 1974 the percentage of fermentation-derived industrial alcohol was approximately 10%; however, this had increased to 30% by 1976. This increased industrial grain alcohol production comes largely from integrated grain milling plants where potable and industrial ethanol is produced among other corn products.

Table 10
COMPARISON OF ATTAINED VS. THEORETICAL
WEIGHT YIELDS ON DEXTROSE

Fermentation products	% Weight conversion yield attained by fermentation	Theoretical yield % (stoichiometry)	Conversion efficiency, %
Anaerobic processes			
Ethanol	46	51.1	90
<i>n</i> -Butanol, H ₂ , acetone, ethanol	29—35	49.8	58—70
2,3 Butylene glycol	45	50	90
Aerobic processes			
Glycerol	43	76.6	56
Fumaric acid	64	64	100

Modified from Tong, G. E., *Chem. Eng. Prog.*, 74, 70, 1978. With permission.

Table 11
CURRENT STATUS OF C₃ AND C₄ CHEMICALS
PRODUCTION

	U.S. 1976 production (million kg)	% Produced by fermentation
Butadiene	1475	0
Acetone	871	5
Isopropanol	780	0
<i>n</i> -Butanol	247	10
Methyl ethyl ketone	237	0
Glycerol	157	0
Maleic anhydride	119	0
Fumaric acid	24	15
2,3 Butylene glycol	—	—
Ethanol total	750	30

Assuming a 34% average weight conversion of carbohydrate to chemicals,¹² the 4 million tons of C₃ and C₄ chemicals can be produced from 12 million tons of starch or other fermentable sugar. The availability of agri-raw materials is not a major problem limiting progress toward fermentation-derived chemicals. This feedstock requirement may be met by expanding the annual cereal grain and sugar crop production by less than 10%, augmented by fermentable sugars in molasses and dairy whey, 0.8 and 0.3 million tons/year, respectively.

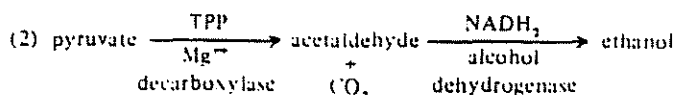
A. Ethanol

In view of continuously rising petroleum costs and dependence upon fossil fuel resources in North America, considerable attention has been focused upon alternative energy resources. Primary consideration involves the production of ethyl alcohol from renewable resources and determination of the economic and technical feasibility of using alcohol as an automotive fuel blended with gasoline. Special emphasis has been directed toward fermentation of surplus grains, agricultural residues, and forest waste

materials as resources for production of alcohol and other chemicals. Since the major production of industrial alcohol is derived synthetically from ethylene, a major technological breakthrough is required to make the fermentation process competitive with that from ethylene.

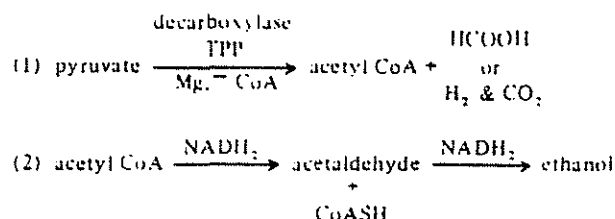
The ability to produce ethanol from glucose is widely distributed among different microorganisms; however, the yields vary considerably from almost 2 mol of ethanol per mol glucose fermented, characteristic of yeast, to much smaller amounts produced by numerous bacteria.¹⁹ These variations are attributable to the operation of four different metabolic routes of ethanol formation, three of which involve pyruvic acid as an obligatory intermediate. Pyruvic acid may be produced from glucose by different metabolic sequences, such as, Embden-Meyerhof glycolysis or Entner-Doudoroff cleavage with subsequent conversion to a C_2 unit via decarboxylation to acetaldehyde, or may be a thioclastic reaction to acetyl coenzyme A. Reduction of either C_2 unit yields ethanol.

1. Type I. Glycolysis



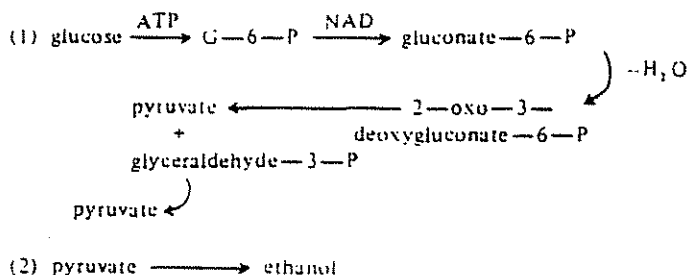
The Type I pathway of microbial metabolism of glucose via pyruvate and acetaldehyde leads to essentially quantitative conversion of glucose to ethanol and carbon dioxide. The yeasts are best known for utilizing this pathway, although bacteria are known that possess a yeast-like pathway and ferment glucose almost quantitatively to ethanol.

2. Type II. Thioclastic Reaction



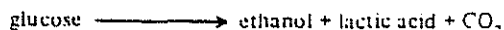
The *Clostridia* and *Enterobacteriaceae* cleave pyruvate to acetyl CoA with subsequent reduction to acetaldehyde and ethanol. For quantitative conversion of glucose to ethanol, H_2 production must be suppressed to provide the reducing power essential for ethanol production.

3. Type III. Entner-Doudoroff Pathway



Zymomonas species give similar fermentation balance to yeast, but ethanol derives from C-2, C-3, C-5, and C-6 of glucose with only one half the energy yield.

4. Type IV. Heterolactic Fermentation



Heterolactic microorganisms are capable of glucose fermentation to lactate and ethanol via xylulose 5- PO_4 , which is subsequently cleaved to yield acetyl PO_4 and glyceraldehyde 3- PO_4 . The latter is converted to pyruvate with subsequent reduction to lactic acid. The acetyl PO_4 is reduced to ethanol, utilizing the reducing power generated from the glucose to xylulose 5- PO_4 conversion.

Conversion of glucose to ethanol by yeast fermentation is well understood in terms of technology and product yield. In defining new possibilities of increasing productivity and reducing distillation costs, very few areas exist in the conventional methods of molasses and starch grains to alcohol. Opportunities exist in strain selection of flocculent yeasts that are tolerant to high sugar concentrations and ferment quickly to around 12% v/v ethanol. The current world production of distilled fermentation alcohol from various substrates is approximately 2.5 million tons/year. It is only in highly industrialized countries that synthetic alcohol from ethylene exists competitively. Trevelyan²⁰ reported the reverse situation in India where fermentation alcohol is used to produce ethylene. The utility of alcohol as a fuel source begins to reflect various economic factors differently as biomass crops for energy production are taken under consideration.

Ethanol production from plant biomass is being studied extensively by various research laboratories throughout the world. Bellamy²¹ and Brooks et al.,²² at General Electric Company, have pursued the production of both single-cell protein (SCP) and alcohol from agricultural wastes by utilizing various biological conversion processes. The process²² involves a steam pretreatment to partially delignify wood and enhance cellulose accessibility to microbial utilization. *Clostridium thermocellum* is utilized to ferment the cellulose directly to ethanol and acetic acid. Research has also been conducted upon thermophilic bacteria that produce ethanol from xylose. Mixed culture fermentation of cellulose to ethanol with thermophilic microorganisms has been evaluated by the General Electric group.

Wang and co-workers^{23,24} (M.I.T.) continue to investigate the cellulolytic activity of mutants of *C. thermocellum* capable of alcohol tolerance to 5% v/v. These organisms generate after 75 hr growth upon cellulose (10 g/l) some 3 g/l reducing sugars and 2 g/l ethanol. *C. thermocellum* grown on corn cob granules consumes from 8 to 66% of the substrate and produces reducing sugars from 1.38 to 2.95 mg/ml.

Research for the past 30 years has mainly concerned the batchwise production of alcohol. However, in recent years considerable work has evolved around continuous fermentation methods. Rosen²⁵ has recently described various continuous fermentations with starchy material or molasses as substrates. The residence times for continuous molasses fermentations are between 7.5 and 13 hr. By using continuous methods, the conversion is increased and the cubic capacity of fermentor vessels is reduced, but also the instrumentation is simplified.

The large increases in crude oil prices in 1973 have stimulated various research projects for the discovery of new energy sources. The nation of Brazil has developed alcohol processes that utilize numerous raw materials that are plentiful in various regions of the country, i.e., cassava roots, palm trees, and sugar cane. The babassu coconut (23% starch), produced at a rate of 210 million tons, can be utilized to produce a wide

variety of products, including charcoal, oil, and alcohol. Considering only the yield of ethanol, theoretically about 8 billion ℓ of ethanol could be produced yearly from the babassu crop in Brazil. This is almost twice the expected production of ethanol in that country, which is estimated at 4.3 billion ℓ by 1980.²⁶

Carioca and Scares,²⁷ experimenting with babassu flour (containing approximately 60% pure starch) as a biomass resource, carried out an alcoholic fermentation. The starch material was gelatinized at 80 to 85°C with subsequent addition of a heat stable α -amylase. Complete saccharification was enhanced by glucoamylase treatment for 40 hr at room temperature. After this hydrolysis procedure, the sugar content measured 9.1%, then pressed yeast and yeast extract nutrients were added. Fermentation was conducted at 28 to 30°C for 42 hr, with subsequent distillation of the mash and redistillation of the initial ethanol product. The yield was 90 ml of 92% purity substance from 250 g crude babassu flour in 1 ℓ distilled H_2O . Based upon yields from 60% starch babassu flour (1 kg), the theoretical yield if all starch were fermented to alcohol, would be 568 g ethanol, a relative yield of 76% in their fermentation process.

The cassava plant has commanded considerable attention recently in Brazil as a starch resource for fermentation.²⁸ Cassava, also known as manioc or tapioca, is characteristically cultivated in many tropical regions of the world for the production of food or animal feed. Cassava, containing 20 to 35% starch and 1 to 2% protein in its roots, is one of the most efficient photosynthesizing plants known. The average crop production in Brazil is 13 tons of roots per hectare. This crop provides a most inexpensive source of starch that is not fully exploited technically for the production of starch products, possibly due to a lack of mechanization in its cultivation and perishability of its roots. The Brazilian Alcohol Program, established in 1975, seeks to utilize 20% ethyl alcohol in gasoline by 1980. To attain this objective, 4.3 billion ℓ of absolute alcohol need to be produced annually by that time. Lindeman and Rocchiccioli²⁸ have discussed in detail the massive plans of the Brazilian government to produce ethanol from sugar cane into 1981. Productivity factors are evaluated with reference to resources, production, and consumption. In 1978, a new cassava alcohol plant began operations in Brazil with a daily output of 60,000 ℓ of absolute alcohol. The feasibility of alcohol production from starch materials to compete with sugar cane will depend principally upon optimization of the liquefaction and saccharification steps of manufacture. These steps are not a requirement for fermentation of cane juice.

Although cassava starch is readily susceptible to α -amylase, the starch granules are weakly bound; thus, the root fiber creates a barrier to the starch hydrolysis if whole cassava roots are used. Rupture of the lignocellulosic components ensures reduction in the slurry viscosity and less energy in cooking, facilitating starch hydrolysis. This fiber removal can be accomplished through biological pretreatment with the cellulolytic *Thermoactinomyces viridae*. Recent work by Menezes et al.²⁹ demonstrated that fungal broths of a Basidiomycete and *T. viridae* increased both the rate of sugar formation and degree of solubilization, with subsequent decrease in slurry viscosity.

In discussion of other potentially useful agricultural wastes, the disposal of whey, a by-product of cheese manufacture, has become a serious pollution problem in many areas. In 1974, some 32.5 billion lb of whey were produced, one half of which was disposed of as waste.³⁰ This biological residue represents some 1.6 million lb of lactose, which can be utilized as a fermentation resource.

O'Leary and co-workers^{31,32} have recently reported alcoholic fermentations of a lactase—hydrolyzed acid whey permeate (4.0 to 4.5% lactose) containing 30 to 35% total solids. Fermentations were conducted for 13 days with *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* with maximal yields of 6.5 and 4.5% ethanol, respectively. Although *S. cerevisiae* converted the available glucose present in the lactase—

hydrolyzed whey permeates to alcohol, the galactose generated was not utilized by the organism. More efficient means and/or organisms will be required to utilize the galactose and glucose to alcohol.

Roland and Alm¹¹ reported that hydrolyzed whey permeate syrups fortified with an N source could be fermented to a 12.5% v/v alcohol beverage with a culture of *S. cerevisiae* var. *ellipsoideus*. Fermentations were conducted, with interval feedings of hydrolyzed whey permeate syrups reaching maximum alcohol in 6 days. Galactose utilization by the yeasts was not measured; however, residual reducing sugars in the wines varied from 0.2 to 4.3%. In summation, a wide variability may exist between the fermentation capacity of *S. cerevisiae* strains to utilize galactose.

The most thoroughly studied process for producing ethanol from biomass is enzymatic conversion of agricultural waste to soluble sugars and subsequent fermentation to ethanol by yeast. Wilke et al.¹⁴⁻¹⁶ and Cysewski and Wilke¹⁷ have provided some preliminary economic evaluations on various principal cost elements. The distribution of costs associated with ethanol production (exclusive of raw material costs) from newsprint and wheat straw by this process is discussed. The major costs of saccharification dominate, because the fermentor capacity required to produce sufficient quantities of fungal cellulase is 30 to 40 times that required to ferment the resulting sugars.

Su and Paulavicius¹⁸ have recently described volumetric production efficiencies for alcohol production by fermentation from newsprint, wheat straw, and molasses. This efficiency in grams per liter per hour is significantly lower than the conventional molasses fermentation by yeast and is reflected in the conversion cost estimates.

Brooks and co-workers,¹² have recently conducted an economic viability study of a process for direct ethanol production from pretreated hardwood chips. These estimates are based upon similarities to the ethanol process from molasses.¹⁹ Cost estimates are based upon a 25 million gal/year-95% ethanol plant from hardwood chips. The first stage involves a high temperature chemical pretreatment followed by a second stage direct fermentation to ethanol. The assumed yield of ethanol was based only on the conversion of the cellulose fraction of the pretreated wood. The conversion of the hemicellulose fraction (~20% of raw material) to ethanol would enhance the overall conversion yield. Utilization of a continuous fermentation process with cell recycle would provide a means of reducing associated costs.

Any conceptual process for saccharification to produce reducing sugars will require feeding of cellulose at high concentrations. Concentrated cellulose slurries are highly viscous and are difficult to pump and stir in conventional agitated fermentors. The mechanical properties of cellulose have been exploited by Wang and co-workers¹⁴ with a packed-bed fermentor with cellulose as stationary phase. The batch packed-bed fermentor consisted of Solka floc cellulose with *Clostridium thermocellum* with liquid recirculation for 48 hr at 60°C. Cellulose degradation was 67%, with 14 g/l total cells adsorbed onto the cellulose bed, compared to cell concentrations of 1 to 2 g/l in typical stirred tank fermentations. The packed-bed technique may well serve as an excellent cell collector where cell recycle can be achieved and high substrate and product concentrations can be attained. Batch packed-bed fermentation by *C. thermocellum* of Solka floc yielded 8 g/l reducing sugar, 2.2 g/l ethanol, and 2.4 g/l acetic acid.

Recent experiments by Kierstan and Bucke²⁰ on immobilized cell technology for alcohol production have been reported with two yeasts. Immobilized treated whole cell preparations have been used primarily in single-step reactions, in particular, in isomerization of glucose. Ethanol production from glucose solutions by an immobilized preparation of *S. cerevisiae* was demonstrated over a total of 23 days, with cell half-life of approximately 10 days. The yeast cells were immobilized in calcium alginate gels.

B. Acetone-Butanol-Isopropanol

Clostridium acetobutylicum historically has been the major organism used for the production of acetone and butanol from starch materials. This fermentation became known as the Weizmann process during World War I. Because of the industrial importance of these compounds, it has been studied in greater detail than other clostridial fermentations. The first stage of the fermentation is essentially butyric and acetic acid accumulation, yielding a pH drop to 4.5, with a second stage utilization of the acids to butanol and acetone with concomitant rise in pH. The butanol is formed by the reduction of butyric acid or butyryl-CoA to the alcohol. Minor quantities of ethanol are produced also in this fermentation.

Clostridium butylicum is an isopropanol type fermentation. The products of this type of clostridial fermentation are similar to acetone-butanol fermentation, except that the acetone is reduced to isopropanol. The extra reduction step normally results in a decrease in the amount of H_2 produced during the fermentation. Significant quantities¹² of acetone and butanol have been produced in the last 10 years in countries such as South Africa, where cheap fermentable biomass is available, but not in fossil fuel dependent countries.

Renewed interest in these fermentations has developed in the area of cellulosic waste conversion to butanol and other oil sparing solvents and chemicals. Recent studies¹¹ on biological production of organic solvents from celluloses involve conversion of animal feedlot residues to liquid fuels. The process plan involves an alkali pretreatment of cattle feedlot residues followed by addition of a high temperature fungus, *Thermoactinomyces* sp., for cellulase production. The third step involves cellulase hydrolysis of the bulk residue with subsequent fermentation of the sugar syrup by *C. acetobutylicum*. Preliminary economic evaluation indicates that, with present knowledge, butanol can be produced for just over 30¢/lb, which is comparable to ethylene based butanol.

Wang and co-workers^{11,14} have recently described significant new research data based upon the *C. acetobutylicum* fermentation. Experiments with a corn meal medium with various strains have been initiated and give every indication that there are strains capable of producing mixed solvents near theoretical maximum yields, i.e., 1.05 and 2.26 g/l for acetone and *N*-butanol, respectively.

C. 2,3-Butanediol (2,3-Butylene Glycol)

A number of facultative anaerobes are characterized by their ability to produce 2,3-butanediol (commonly called 2,3-butylene glycol). In general, 2,3-butanediol, produced by species of *Klebsiella*, *Bacillus*, and *Serratia* is a major fermentation product; however, in the presence of air, the oxidation product acetylmethyl carbinol is formed instead. Butanediol is important industrially as a potential raw material for synthetic rubber and was heavily investigated during World War II.

In the butanediol fermentation, glucose is broken down to pyruvic acid, which is further metabolized to butanediol. Although the major amounts of butanediol are produced by bacteria, yeasts form minor amounts of butanediol.¹⁵ *Bacillus subtilis*, *Aerobacter aerogenes*, and *Serratia marcescens* produce significant quantities of butanediol from acid hydrolyzed starch,¹⁵ some 35 lb butanediol/100 lb starch. Early investigations by Perlman¹⁶ involved the production of 2,3-butanediol from acid hydrolyzates of hard and soft woods. *Aerobacter aerogenes* fermentations yielded from 24 to 30% butanediol depending upon the type of wood utilized.

D. Propionic Acid

Propionic acid is a major end-product of glucose fermentation in *Propionibacterium*

species, occurring also with acetic acid and CO_2 . The fermentation involves the reduction of two pyruvic acid molecules to propionic acid, with the oxidation of a third molecule to acetic acid and CO_2 .

Recent research has been conducted on the bioconversion of propionic acid to acrylic acid by *Clostridium propionicum* from renewable resources.⁴¹⁻⁴⁵ Acrylic acid is a high-volume industrial chemical in high demand (approximately 1 billion lb/year). Two anaerobic organisms, *Peptostreptococcus elsdenii* and *Clostridium propionicum*, accumulate this acid as an intermediate. In *C. propionicum*, lactate is converted to acrylate, then to propionate via activated CoA thio esters. In resting cells of *C. propionicum*, propionate is oxidized to acrylate in the presence of an electron acceptor such as O_2 or methylene blue. Acrylate production is stimulated by sodium lactate. Concentrations of acrylic acid in excess of 4 mM have been achieved with resting cells.

E. Glycerol-Succinic Acid

Oura⁴² discusses in detail the formation of glycerol and succinate by yeasts. The formation of glycerol appears to be nonphysiological, and quite useless for the yeast cell that obtains neither energy nor building units from it.⁴⁶ During the fermentation of glucose by yeast at pH 6 or below, only small amounts of glycerol are formed. Addition of sulfite to the medium increased glycerol production severalfold. This fermentation is known as the Neuberg 2nd and 3rd forms, in which glycerol accumulates in the fermentation. Two oxidation steps are involved in glycerol formation from glucose, and the redox balance will be achieved by the formation of two units of glycerol. Apparently, there is a direct correlation between redox balance of the cell and the formation of glycerol. When yeasts metabolize glucose under aerobic conditions, no superfluous glycerol is formed. Under these circumstances, the respiratory chain is functioning and transfers electrons to O_2 with no excess of NADH_2 .

Two mechanisms have been proposed for the formation of succinate in yeast during anaerobic fermentations. One is formation via the normal oxidative mechanism of the TCA cycle, and the other is via a reductive pathway with malate and fumarate as intermediates.^{47,48}

The formation of succinate is considerably lower during anaerobic growth than during fermentation, and the physiological state of the cell is different in these two cases. The level of energy-rich nucleotides during growth is low, whereas the energy charge increases strongly during yeast fermentation. The activity of many anabolic ATP dependent enzymes is modified by the energy charge of the cell, such as pyruvate carboxylase.⁴⁹ When energy charge is high (during fermentation), pyruvate is metabolized to oxalacetate via an activated pyruvate carboxylase, and the TCA cycle will function actively. The cycle intermediates accumulate as succinate and are excreted into the medium.

Therefore, since fermentation leads to an elevated energy charge in the cell (pyruvate-carboxylase activation), formation of succinate occurs and an excess of reduced respiratory nucleotides. This excessive NADH_2 is oxidized in the formation of glycerol, yielding a balance in the redox state of the cell.

F. Acetic Acid

Although numerous organisms are capable of a nonphosphorylative glucose oxidation to acetic acids, recent findings with some anaerobic organisms have stimulated interest in acetic acid production. The anaerobic cellulolytic rumen bacterium *Ruminococcus flavefaciens* normally produces succinic acid as a major fermentation product with acetic and formic acids, H_2 and CO_2 . When grown on cellulose and in the presence of the methanogenic rumen bacterium *Methanobacterium ruminantium*, ace-

tate was the major fermentation product. This type of interaction may be of significance in determining the flow of cellulose carbon to the normal rumen fermentation products.

Baleh et al.¹⁰ recently described a new genus of fastidiously anaerobic bacteria that produce a homoacetic acid fermentation. The type species, *Acetobacterium woodii*, ferments fructose, glucose, lactate, glycerate, and formate. Hydrogen is oxidized and CO₂ is reduced to acetic acid. Schoberth¹¹ has demonstrated the formation of acetate by cell extracts of *Acetobacterium woodii*.

Wang and co-workers¹² have recently reported studies on ethanol and acetic acid production by the cellulolytic anaerobe, *Clostridium thermocellum*, on cellulosic biomass. Experiments were conducted in cellulose packed-bed fermentors. Cellulose degradation was 67%, with a yield of 2.4 g/l acetic acid from 110 g/l cellulose.

Brooks and co-workers¹³ have described a mixed culture fermentation of cellulose (microcrystalline) at 55°C that yielded acetic acid as the major organic chemical produced, plus ethanol, 2,3-butanediol, and CO₂. This group has also studied a continuous fermentation of a thermophilic *Bacillus* that produced ethanol and acetic acid at various dilution rates.

G. Fumaric Acid

Fumaric acid is produced principally by the fermentation of glucose or molasses with species of the genus *Rhizopus*. Rhodes et al.¹⁴ reported fumaric acid yields of 60 to 70% in 3 to 8 days in shaken flasks containing 10 to 16% glucose or sucrose, or the partially inverted sucrose of molasses. Although fumaric can be produced in high yields by fermentation, it is produced commercially as a by-product in the manufacture of phthalic and malic anhydrides or by isomerization of malic acid with heat and catalyst. A number of chemicals can be produced from fumaric acid, including malic acid, coumaric acid, and maleic anhydride.

H. Citric Acid

The manufacture of citric acid is conducted presently by fermentation of sugar-containing material by microorganisms of the species *Aspergillus niger*. Both surface and submerged fermentation have been utilized for production of 70 kg of citric acid/100 kg of sugar content of raw material (usually molasses).

Usami and Fukutomi¹⁵ recently reported on a citric acid solid fermentation by *A. niger*, sugar cane molasses, and pineapple molasses. After 3 days, 50 to 60% citric acid yield per equivalent sugar was available.

Hang et al.¹⁶ reported upon the production of citric acid by *A. foetidus* from spent grain liquor, a brewery waste. The yields of citric acid varied from 3.5 to 12.3 g/l of the waste fermented. Methanol addition (2 to 4%) markedly increased the formation of citric acid from wastes.

The citric acid-producing fungi can thus be utilized not only for organic chemical production but also for converting the BOD of brewery wastes into fungal protein.

I. Lactic Acid

Wastes from the pulp, paper, and fiberboard industries contain considerable sugar polymers, and thus present a high BOD to receiving waters. Griffith and Compare¹⁷ describe a fixed-film system for continuous lactic acid production from waste waters. Lactic acid yield is in excess of 50%, the carbohydrate is available and readily recovered. The fixed-film unit (2 in. × 6 ft) was seeded with lactobacilli and lactose fermenting yeasts (kefir culture). The wood molasses substrate was pretreated with cellulases, a diastase, and hemicellulases. With a feed rate of 60 g/l wood molasses, 31 to 32 g/l lactic acid yields were obtained.

The production of calcium lactate from molasses by *Lactobacillus delbrueckii* was studied by Tewari and Vyas⁵⁶ using different growth factors from moong sprouts and various oil seed cakes. Maximum conversion of molasses plus 5% moong sprouts was achieved within 7 days at 50°C.

J. Malic Acid

Pichia membranaefaciens is capable of converting fumaric acid to L-malic acid. In a recent report, Takao and HoHa⁵⁷ describe malic acid yields as high as 80% or more, based on initial glucose when *Rhizopus arrhizus* (fumaric acid production) was grown 2 to 3 days and then associated with *Proteus vulgaris*. Malic acid formation also occurred when *R. arrhizus* was grown in mixed culture.

K. Methanol

Methanol occurs in nature as a breakdown product during microbial decomposition of plant materials and as a metabolite of methane-utilizing bacteria during growth upon methane or natural gas. Foo⁵⁸ recently reviewed some of the basic considerations in search of microorganisms with potential for microbial production of methanol. No attempt will be made to discuss the voluminous literature relative to the microbial production of methanol.

Since petroleum feedstocks are no longer cheap (as in the early 1950s), production of liquid fuels via fermentation has gained wide attention, especially alcohol fuels. In recent years, methanol has become a potentially important carbon source for the production of SCP, enzymes, and amino acids.⁵⁹ Methanol is also a potential fuel for internal combustion engines, since it possesses cleaner burning properties and produces less pollution than hydrocarbon fuels. A large volume of methanol is used as a solvent and as an intermediate in chemical manufacture.

Methanol can be produced by the destructive distillation of wood; however, most methanol is derived from carbon monoxide with hydrogen reaction.⁶⁰ In nature, methanol arises from the breakdown of methyl esters and/or ethers from decomposition of pectin-like plant materials. Very little is known about the microorganisms that produce methanol during decomposition of organic material; however, numerous reviews are available.^{61,62}

Methanol inhibition, and the energy and reducing power requirements of methane oxidation present major problems to the excretion of excess methanol by microorganisms. Only small amounts of methanol are excreted by the cell biomass yields of methanol-utilizers in mixed culture studies.^{63,64} Greater tolerance is needed to improve yields of methanol and further productivity under possibly elevated pressure. Greater numbers of methane-utilizers will have to be isolated and tested in order to find those more suited to methanol excretion.

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